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Inorganica Chimica Acta 279 (1998) 51–57

Inorganica Chimica Acta

Intercalator-linked cisplatin: synthesis and antitumor activity of *cis*-dichloroplatinum(II) complexes connected to acridine and phenylquinolines by one methylene chain

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Received 9 September 1997; received in revised form 17 October 1997; accepted 4 December 1997

Abstract

Three novel intercalator-linked cisplatin-type platinum complexes, *cis*-[$PtCl_2$(9-(2-aminoethyl)aminomethylacridine)] (**1**), *cis*-[$PtCl_2$(4-(2-aminoethyl)aminomethyl-2-phenylquinoline)] (**2**), and *cis*-[$PtCl_2$(8-(2-aminoethyl)aminomethyl-2-phenylquinoline)] (**3**) were synthesized. The structure of **1** was determined by X-ray crystallography (triclinic, space group $P\bar{1}$ with $a=15.007(6)$, $b=15.597(4)$, $c=10.398(3)$ Å, $\alpha=98.51(3)°$, $\beta=96.79(3)°$, $\gamma=114.61(2)°$, $Z=4$, $R=0.053$, $R_w=0.063$). The antitumor activity of the platinum complexes was investigated against the HeLa cell. Compound **3** was the most cytotoxic among the complexes synthesized here and was more effective than cisplatin. It was suggested from microscopic analysis that the acridine complex **1**, which had no cytotoxicity against the HeLa cell, was not incorporated in the nucleus of the cell. Against the P388 cell, however, complex **1** gave a more therapeutic result than **3**. The covalent binding ability of the cisplatin moiety was suppressed significantly in these compounds. The results of molecular mechanics showed that intercalation and covalent binding could be compatible. The cytotoxicity and DNA binding ability of phenylquinoline-type ligands were also studied to evaluate the intrinsic cytotoxicity of the intercalator. From the duplex DNA denaturation experiment and fluorescent ethidium displacement assay, the DNA binding affinities of the ligands are in agreement with the cytotoxicity of these compounds and the corresponding platinum complexes.

Keywords: Platinum complexes; Acridine complexes; Crystal structures; Antitumour activity

1. Introduction

One of the most serious problems in cancer chemotherapy is the treatment of solid tumors. DNA-binding agents which have reversible binding affinity should spread to a broad sphere of tumor tissues [1–3]. Since the strong affinity of a drug to DNA seems to prevent dispersion of the drug, a molecule with moderate binding ability might be effective. Among the many DNA-intercalators explored up to now, phenylquinoline [4,5], phenylbenzimidazole [6,7], and dinitrobenzene [8] derivatives have been reported to have considerable possibilities. Intercalative binding is not the only requirement for anticancer drugs, so it is interesting to attempt to introduce a second function to these molecules.

Anticancer platinum drugs have been studied extensively because cisplatin has a significant effect on testicular cancer and a wide variety of solid tumors [9,10]. The covalent binding of cisplatin to purine (particularly guanine) bases of DNA is considered to induce the antitumor activity. In addition to the formation of the DNA–cisplatin–intercalator ternary complex [11,12], many cisplatin derivatives connected to a DNA-binding motif have been investigated to improve the efficacy of platinum drugs towards other cancers and drug-resistant tumors [13–18].

We report here the preparation and biological activities of some platinum complexes tethered to an intercalator such as acridine or 2-phenylquinolines by one methylene chain. The short linkage is expected to retard the covalent binding of the cisplatin moiety, and these complexes might be delivered to the core of solid tumor masses. In this report, the synthesis, structure determination, permeability into cells, interactions

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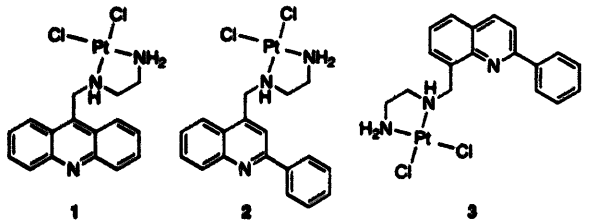
0020-1693/98/$ - see front matter
PII S0020-1693(98)00035-8

with DNA, and antitumor activity against the HeLa and P388 cells for these complexes were studied. The cytotoxicity and DNA binding abilities of the ligand itself were also studied to understand the biological data. A preliminary account of this work has already been published [19].



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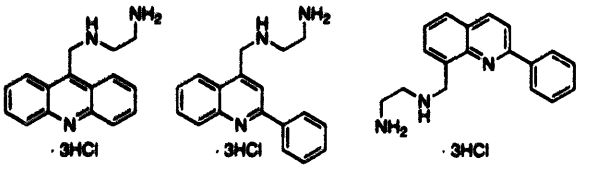

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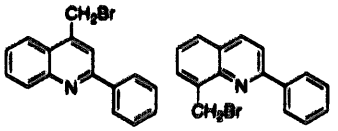

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2. Experimental

2.1. General

Melting points were determined on a micro hot-stage Yanaco MP-S3. ^{1}H NMR spectra were measured on a Varian GEMINI 2000 spectrometer at 300 MHz. Absorbance spectra and DNA denaturation experiments were run on a Shimadzu UV-3100PC with an SPR-8 temperature controller. Cell counting by the MTT method was carried out on a BIORAD model 550 microplate reader with 550 nm band path filter. Fluorescence measurements were performed on a Jasco FP-750 with ETC-272T temperature controller.

2.2. Materials

Calf thymus DNA and the other oligodeoxynucleotides were obtained from SIGMA and used without purification.

2.2.1. 9-(2-Aminoethyl)aminomethylacridine trihydrochloride (4)

To a stirred solution of ethylenediamine (1.00 g, 16.7 mmol) in anhydrous acetonitrile (20 ml), 1.12 g (4.1 mmol) of 9-bromomethylacridine [20] in 100 ml of anhydrous hot acetonitrile was added dropwise at room temperature. The mixture was stirred overnight. After removal of insoluble material by filtration, the solvent was removed in vacuo. The obtained yellow oil was dissolved in 100 ml of 0.2 N HCl and washed with dichloromethane. The aqueous solution was treated with 5 N NaOH to pH 8–9 and extracted with dichloromethane three times. The extracts were dried (Na_2SO_4) and evaporated to give pale yellow oil. The obtained free amine was dissolved in the smallest amount of conc. HCl, an appropriate amount of EtOH was added, and the solution was cooled in a refrigerator to give a yellow powder (0.61 g, 38%). ^{1}H NMR (DSS) in D_2O: δ 3.45 (t, 2H), 3.73 (t, 2H), 5.58 (s, 2H), 8.1 (dd, 1H), 8.3–8.5 (m, 2H), 8.74 (d, 1H). MS: *m/z* 252 ($M+H^+$). *Anal.* Calc. for **4**·$2H_2O$, $C_{16}H_{24}N_3O_2Cl_3$: C, 48.43; H, 6.09; N, 10.59. Found: C, 48.38; H, 6.26; N, 10.65%.

2.2.2. 4-Bromomethyl-2-phenylquinoline (5)

A mixture of 4-methyl-2-phenylquinoline [21] (0.40 g, 1.8 mmol), *N*-bromosuccinimide (NBS) (0.38 g, 2.1 mmol), and 0.02 g of 2,2′-azobisisobutyronitrile (AIBN) in 30 ml of carbon tetrachloride was refluxed for 4 h. After cooling the reaction solution to room temperature, insoluble materials were filtered and the solvent was removed in vacuo. The resulting residue was subjected to silica gel column chromatography (petroleum ether:dichloromethane = 1:1 as an eluent) to give a white solid (0.29 g, 54% yield), m.p. 89–91°C. ^{1}H NMR (Me_4Si) in $CDCl_3$: δ 4.92 (s, 2H), 7.45–7.58 (m, 3H), 7.63 (t, 1H), 7.76 (t, 1H), 7.91 (s, 1H), 8.1–8.26 (m, 4H).

2.2.3. 4-(2-Aminoethyl)aminomethyl-2-phenylquinoline trihydrochloride (6)

From **5**, a procedure similar to that used for the preparation of **4** gave **6** in 76% yield, m.p. 170–173°C. ^{1}H NMR (DSS) in D_2O: δ 3.54 (t, 2H), 3.70 (t, 2H), 5.08 (s, 2H), 7.7–7.8 (m, 3H), 7.97 (t, 1H), 8.06–8.2 (m, 3H), 8.3–8.4 (m, 3H).

2.2.4. 8-Bromomethyl-2-phenylquinoline (7)

Bromination of 8-methyl-2-phenylquinoline [5] was run in a similar manner as for the preparation of **5**, using hexane/ether = 9/1 as an eluent for silica gel column chromatography (96% yield), m.p. 83–85°C (literature value [22] 78% yield, m.p. 87–87.5°C). ^{1}H NMR (Me_4Si) in $CDCl_3$: δ 1.52 (s, 1H), 5.35 (s, 2H), 7.25 (s, 1H), 7.48–7.65 (m, 4H), 7.79–7.88 (m, 2H), 7.95 (d, 1H), 8.2–8.45 (m, 3H).

2.2.5. 8-(2-Aminoethyl)aminomethyl-2-phenylquinoline trihydrochloride (8)

The treatment of **7** with ethylenediamine gave **8** in accordance with the preparation of **4** (24% yield), m.p. 205–207°C. ^{1}H NMR (DSS) in D_2O: δ 3.25–3.35 (m, 2H), 3.4–3.5 (m, 2H), 7.45–7.6 (m, 4H), 7.76 (d, 1H), 7.88–7.98 (m, 2H), 8.05 (d, 2H), 8.28 (d, 1H). *Anal.* Calc. for $C_{18}H_{22}N_3Cl_3$: C, 55.90; H, 5.73; N, 10.86. Found: C, 55.41; H, 5.80; N, 10.50%.

2.2.6. *cis*-[$PtCl_2$(9-(2-aminoethyl)aminomethylacridine)] (1)

The compound **4** (0.397 g, 1.0 mmol) was dissolved in water and the pH was adjusted to 9 with KOH. To an aqueous solution (4 ml) of $K_2[PtCl_4]$ (0.415 g, 1.0 mmol), ligand solution was added dropwise and the mixture was stirred for 2 h at room temperature. The reaction mixture was kept in a refrigerator overnight and the resulting orange precipitate was collected with suction, washed with water and dried in vacuo. The crude product recrystallized from DMF afforded orange plates (0.15 g, 29% yield), m.p. 216–218°C (dec). *Anal.* Calc. for $C_{16}H_{17}N_3Cl_2Pt$: C, 37.15; H, 3.31; N, 8.12. Found: C, 37.09; H, 3.79; N, 8.03%.

2.2.7. *cis*-[$PtCl_2$(4-(2-aminoethyl)aminomethyl-2-phenylquinoline)] (2)

A similar procedure to that used for the synthesis of **1** gave **2** in 21% yield, m.p. > 300°C. *Anal.* Calc. for $C_{18}H_{19}N_3Cl_2Pt$: C, 39.79; H, 3.52; N, 7.73. Found: C, 39.60; H, 3.42; N, 7.45%.

2.2.8. *cis*-[$PtCl_2$(8-(2-aminoethyl)aminomethyl-2-phenylquinoline)] (3)

A similar procedure to that used for the synthesis of **1** gave **3** in 40% yield, m.p. 266–270°C. *Anal.* Calc. for $C_{18}H_{19}N_3Cl_2Pt$: C, 39.79; H, 3.52; N, 7.73; Cl, 13.05. Found: C, 40.09; H, 3.59; N, 7.64; Cl, 12.75%.

2.3. X-ray crystallographic analysis of 1

Suitable crystals for X-ray crystallography were obtained by a recrystallization of **1** from dimethylformamide. The crystal data and the experimental conditions are listed in Table 1. All data were collected on a Rigaku AFC7R diffractometer using graphite-monochromatized Mo Kα (λ = 0.71069 Å) radiation. Three standard reflections were monitored every 150 reflections and no systematic decrease in intensity was observed. Reflection data were corrected for Lorentz-polarization and absorption effects (by the ψ-scan method). The structure of **1** was solved by direct methods with SHELEX 86 [23]. Hydrogen atoms are included but not refined. The structure was refined with full-matrix least-square techniques minimizing $\Sigma w(|F_o| - |F_c|)^2$. Final refinement with anisotropic thermal parameters for non-hydrogen atoms converged to $R = 0.053$ and $R_w = 0.063$. Atomic scattering factors f' and f'' for Pt, O, N, and C were taken from the literature [24,25]. All calculations were carried out on a Silicon Graphics Indigo2 workstation with the TEXSAN program [26]. Perspective drawings were constructed using the ORTEP program [27].

Table 1
Crystallographic and experimental data for **1**·DMF

Formula	$C_{19}H_{24}N_4OCl_2Pt$
FW	590.42
Crystal size (mm)	0.16×0.12×0.03
Crystal system	triclinic
Space group	$P\bar{1}$
a (Å)	15.007(6)
b (Å)	15.597(4)
c (Å)	10.398(3)
α (°)	98.51(3)
β (°)	96.79(3)
γ (°)	114.61(2)
V (Å^3)	2144(1)
Z	4
T (°C)	20
D_{calc} (g cm^{-1})	1.829
D_{meas} (g cm^{-1})	1.83
Absorption coefficient (cm^{-1})	67.8
Transmission factor	0.4838–0.9990
2θ range (°)	$3 < 2\theta < 55$
No. unique data	9864
No. observed data	5421 ($I > 3\sigma(I)$)
No. variables	487
R [a]	0.053
R_w [b]	0.063
Goodness of fit	0.74

[a] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
[b] $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$ ($w = 1/\sigma^2(F_o)$).

2.4. Cytotoxicity against the HeLa cell

10^3 Human uterine cancer cells (HeLa cells) were incubated in the growth medium (200 μl of minimum essential medium (MEM) contains 10% of bovine fetus serum) at 37°C overnight. The incubation was continued in the presence of drugs for more than 72 h and the number of surviving cells was analyzed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [28].

2.5. Antitumor activity against the P388 cell in mice

10^6 P388 cells were transplanted intraperitoneally (ip) into CDF_1 mice (6 mice per group), then the drugs were given by the ip method two times on days 1 and 5. The mean survival time of the treated group (T) was compared with that of the untreated control group (C).

2.6. DNA denaturation experiment

Absorbance versus temperature melting curves were measured at 260 nm using 5×10^{-5} M (in base pairs) of calf thymus DNA in 1.0 mM sodium cacodylate buffer containing 4.0 mM NaCl (pH 6.0). The heating rate was 3°C min^{-1}. T_m values were determined from first derivative plots of melting curves.

2.7. Fluorescent ethidium displacement assay

C_{50} values (the micromolar drug concentration necessary to reduce the fluorescence of initially DNA-bound ethidium by 50%) were obtained using 0.5 μM (in base pairs) of DNA (1.0 mM sodium cacodylate buffer containing 4.0 mM NaCl (pH 6.0)) with 1.26 μM of ethidium at 25°C. The extent of

fluorescence quenching due to non-displacement mechanisms was estimated by similar methods employing 20 μM of DNA and 2 μM of ethidium [29].

2.8. Molecular mechanics

The BIOGRAF (version 3.21) with Dreiding II force field was used to compare the energy of various conformers of the platinum complex-modified oligonucleotide, d(CCU^{Br}-CTG*G*TCTCC)–(GGAGACCAGAGG) (G* denotes platinated guanine) on a TITAN 3000 workstation [30,31]. The starting atomic coordinate of the oligodeoxynucleotide was obtained from the X-ray crystallographic data for the cisplatin-modified oligodeoxynucleotide in the protein data bank [32]. The bond distances and the angles for the coordination of four nitrogen atoms to the platinum(II) were fixed. Because most platinum(II) complexes have rigid and planar coordination structures, this approximation will not result in a large error in the relative energy of the conformers examined.

3. Results and discussion

Intercalator-linked ethylenediamine ligands **4**, **6**, and **8** were synthesized from corresponding methylacridine or methyl-2-phenylquinolines. The condensation of diphenylamine with acetic acid was the most convenient procedure for obtaining 9-methylacridine [33]. 8-Methyl-2-phenylquinoline [5] and 4-methyl-2-phenylquinoline [20] were also synthesized by literature methods. The methyl groups of these compounds were converted to the 2-aminoethylaminomethyl group via bromination by NBS and treatment with ethylenediamine, in yields of 33 (**4**), 41 (**6**), and 17% (**8**). These ligands were reacted with K_2PtCl_4 in water to give the corresponding dichloroplatinum complexes in 21–44% yields.

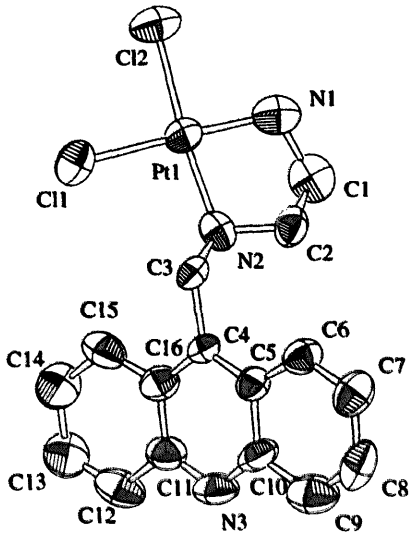

Fig. 1. ORTEP drawing for one of the two independent platinum complexes **1**.

Recrystallization of **1** from DMF afforded single crystals suitable for X-ray crystallography. The elucidated structure is shown in Fig. 1. The asymmetric unit contains two crystallographically independent complexes, their structures being almost identical with each other. Selected bond lengths and angles are listed in Table 2. The coordination around platinum is almost square planar, as for the related platinum complexes reported earlier [13]. The Pt–N and Pt–Cl distances are also typical for the *cis*-diaminodichloroplatinum(II) complex. Intermolecular π–π stacking interaction and hydrogen-bond association between the terminal aliphatic nitrogen atom and the chlorine atom were observed.

The complexes were assayed with the HeLa cell to estimate their cytotoxicity. The results are summarized in Table 3, and indicate that the acridine complex **1** has no toxicity against the HeLa cell. Fluorescent microscopic analysis evidenced no fluorescence from the nucleus of the cell incubated in the

Table 2
Selected bond distances (Å) and angles (°) for **1**·DMF [a]

Bond distances							
	atom	atom	distance		atom	atom	distance
	Pt1	Cl1	2.298(5)		Pt2	Cl3	2.313(4)
	Pt1	Cl2	2.313(5)		Pt2	Cl4	2.318(4)
	Pt1	N1	2.02(2)		Pt2	N4	2.01(1)
	Pt1	N2	2.05(2)		Pt2	N5	2.07(1)
Bond angles							
atom	atom	atom	angle	atom	atom	atom	angle
Cl1	Pt1	Cl2	91.8(2)	Cl3	Pt2	Cl4	92.2(2)
Cl1	Pt1	N1	177.4(5)	Cl3	Pt2	N4	177.0(4)
Cl1	Pt1	N2	94.2(4)	Cl3	Pt2	N5	94.2(4)
Cl2	Pt1	N1	90.5(5)	Cl4	Pt2	N4	90.1(4)
Cl2	Pt1	N2	174.0(4)	Cl4	Pt2	N5	173.6(4)
N1	Pt1	N2	83.5(6)	N4	Pt2	N5	83.6(5)
Pt1	N1	C1	110(1)	Pt2	N4	C17	109(1)
Pt1	N2	C2	107(1)	Pt2	N5	C18	107.1(9)
Pt1	N2	C3	120(1)	Pt2	N5	C19	118(1)

[a] Estimated deviations are given in parentheses.

Table 3
Cytotoxicity of platinum complexes and ligand molecules against the HeLa cell

Drug concentration (M)	Cell survival (%)						
	1	2	3	cisplatin	4	6	8
1.0×10^{-7}	–	99.1	100.0	100.0	–	97.0	91.3
1.0×10^{-6}	–	74.3	58.3	70.6	–	73.0	61.9
1.0×10^{-5}	–	69.7	14.7	29.6	–	76.5	16.2
1.0×10^{-4}	–	0.3	1.8	2.8	–	1.1	1.0

Table 4
Antitumor activity (T/C (%)) of platinum complexes **1** and **3**, and ligand **8** against the P388 cell in mice

Drug	Dose (mg kg^{-1})					
	12.5	25	50	100	200	400
1	103	107	113	110	113	113
3	108	100	100	100	104	100
8	–	–	110	111	–	0[a]

[a] More than half the mice died before day 5.

presence of **1**. These results indicate that this complex was not incorporated into the nucleus of the HeLa cell. The phenylquinoline complexes, however, were clearly cytotoxic. Complex **3** was more cytotoxic than cisplatin under the present experimental conditions. Although the microscopic analyses failed owing to the fluorescent inactivity of complexes **2** and **3**, 2-phenylquinoline seems to be superior to acridine with respect to membrane permeability into the HeLa cell.

The antitumor activities of the complexes **1** and **3** and the ligand **8** against the P388 cell in mice were also examined. Table 4 demonstrates the pharmacological effect of **1**. The effect was very small but there is a distinct activity (T/C = 113%). It should be noted that the toxicity of the ligand molecule **8** in higher doses was masked by the complexation with the platinum atom. These two platinum complexes have no toxicity even at the highest dose (400 mg kg^{-1}).

The intercalator-linked platinum complexes treated in this study have a short spacer. Therefore, it is expected that the formation of covalent bonds of the cisplatin moiety in these complexes might be very slow. The UV–Vis spectrum of complex **1** (2.5×10^{-5} M) dissolved in water containing 25% DMF in the presence of calf thymus DNA (200 μM) was unchanged for several hours. After 24 h, the absorbances of the visible region due to the chromophore of the complex were completely lost: all the complexes were precipitated. If covalent binding of the drug with DNA occurs, the complex would be solubilized and remain in solution. This complex precipitates before covalent bond formation, since the rate of covalent bond formation is fairly slow. Thus, these platinum complexes are insoluble in water and slightly soluble in organic solvents containing water, and covalent binding to DNA has not yet been shown. Modification of the complex is necessary for further investigation.

The crystal structure of cisplatin-modified oligodeoxynucleotide has been reported by Takahara et al. [32]. The unwinding and bending induced by platination were clarified in detail. Using the atomic coordinate of this crosslinked DNA, we examined the compatibility of the covalent crosslinking and the non-covalent intercalation of the acridine moiety in the present platinum complex **1** by molecular mechanics. The results are shown in Fig. 2, representing the two binding modes, e.g. non-intercalative and intercalative models. In the latter case, energy minimization resulted in unpaired bases and a translocated G–C base pair in the platinated GG sequence. However, the relative energy of these

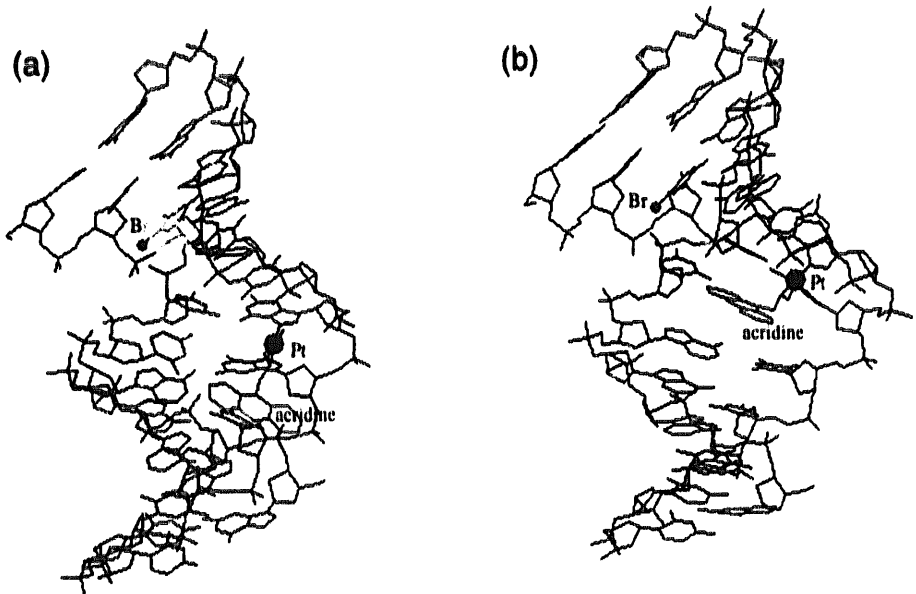

Fig. 2. Molecular modeling of intrastrand crosslinking of d(CCU^{Br}CTG*G*TCTCC)–(GGAGACCAGAGG) (G* denotes platinated guanine) with **1**: (a) non-intercalation model, (b) intercalation model.

two binding models was almost identical in spite of the additional deformation of the double strand in the intercalation model. This result suggests that the oligodeoxynucleotide is flexible enough to allow the acridine moiety to intercalate, maintaining the GG intrastrand crosslink with the platinum complex, and that the suppression of covalent bonding is not a thermodynamic but a kinetic property of the system.

As shown in Table 3, the 2-phenylquinoline ligands **6** and **8** exhibited considerable cytotoxicity whereas the acridine ligand **4** did not. The inactivity of **4** was explained by its poor membrane permeability into the nucleus, as is suggested by fluorescent microscopic analyses. To investigate the difference in activity between **6** and **8**, the DNA binding ability of these ligand molecules was studied. UV–Vis and emission titration of the acridine series (**1** and **4**) with calf thymus DNA have already been reported [19]. The DNA binding abilities of the ligand **4** ($K_{ass} = 3.4 \times 10^4\ M^{-1}$) and the Pt-complex **1** ($K_{ass} = 4.3 \times 10^4\ M^{-1}$), obtained by fluorescent DNA titration in water containing 25% DMF, were almost identical.

Fig. 3 shows the UV-melting curves of calf thymus DNA, demonstrating the enhanced thermal stability of the duplex in the presence of **6** and **8**. Increasing the concentration of both ligands led to amplified stabilization of the duplex. The difference in thermal denaturation temperatures (ΔT_m) is summarized in Table 5. The difference in position of the side chain to the chromophore affected the result significantly. Compound **8** stabilizes duplex DNA more strongly than **6**, in agreement with the result of cytotoxicity against the HeLa cell. Since these compounds are considered to penetrate into the cell nucleus, their cytotoxicity seems to be influenced by the DNA binding ability [1].

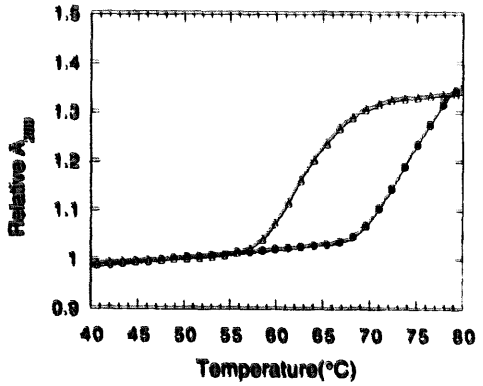

Fig. 3. UV melting profiles of calf thymus DNA in the presence of 2-phenylquinoline derivatives **6** (△) and **8** (●) at a [drug]/[DNA] ratio r of 0.5.

Table 5
Difference in melting temperature (ΔT_m) of calf thymus DNA in the presence of 2-phenylquinoline derivatives [a]

Compound	ΔT_m (°C)		
	$r = 0.1$	$r = 0.3$	$r = 0.5$
6	3.5	6.5	7.6
8	8.7	14.2	17.7

[a] r = [drug]/[DNA].

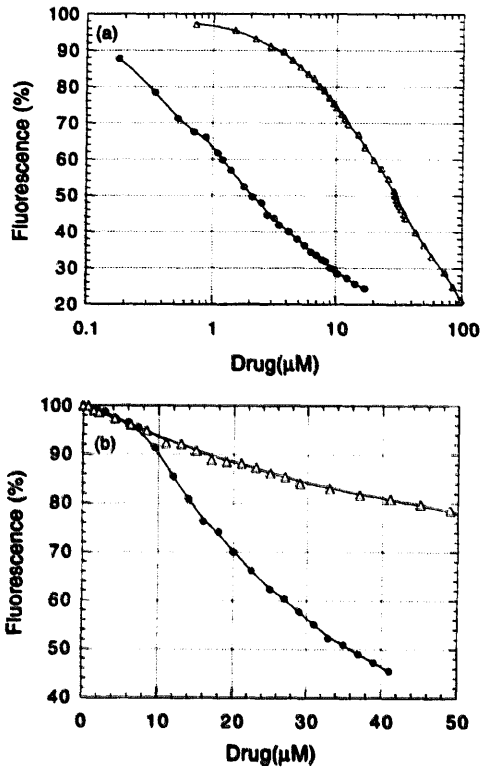

Fig. 4. Decrease in fluorescence of DNA-bound ethidium following addition of 2-phenylquinoline derivatives **6** (△) and **8** (●): (a) [DNA] = 0.5 μM, [ethidium] = 1.26 μM; (b) [DNA] = 20 μM, [ethidium] = 2 μM.

Table 6
C_{50} values in the ethidium displacement assay bound to calf thymus DNA, poly[d(AT)]$_2$, and poly[d(GC)]$_2$ [a]

Compound	C_{50} (μM)		
	Calf thymus	Poly[d(AT)]$_2$	Poly[d(GC)]$_2$
6	30	33	48
8	2.1	5.2	5.6

[a] [DNA] = 0.5 μM, [ethidium] = 1.26 μM.

Fig. 4(a) shows the fluorescence quenching of ethidium bound to calf thymus DNA on the addition of compounds **6** and **8**. The micromolar drug concentrations necessary to reduce the fluorescence of initially DNA-bound ethidium by 50% (C_{50} values) are summarized in Table 6. The apparent quenching is caused by the displacement of ethidium and the quenching of DNA-bound ethidium by intercalated ligands. Since fluorescence quenching by non-displacement mechanisms was ascertained to be small (~5%) as shown in Fig. 4(b), where 20 μM of DNA and 2 μM of ethidium were

[1] In fact, during the investigation of other isomers which have the 2-aminoethylaminomethyl side chain in 7- and 4'- (para position of the 2-phenyl ring) positions, the activity against the HeLa cell and DNA binding ability of these compounds were well correlated (Bioorg. Med. Chem. Lett. (1998) in press).

used, the C_{50} values obtained here are regarded as a direct measurement of the strength of DNA binding of the compounds in this condition. The values are parallel to the results of cytotoxicity and DNA-melting experiments, i.e. the cytotoxic compound binds to DNA strongly.

Complexes **2** and **3** show similar cytotoxicity to the corresponding ligands. It could be considered that this cytotoxicity is due to the chromophore and the platinum function is completely masked. Although the ability of these platinum drugs to bind covalently to DNA has not been evidenced, it is safely concluded that the covalent binding ability of the cisplatin moiety was suppressed significantly in these compounds. Nevertheless, **2** and **3** were satisfactorily taken into the cell and these drugs that accumulated inside the cell showed remarkable cytotoxicity and some pharmacological effects. Determination of the main function of these complexes requires syntheses of defunctionalized compounds, i.e. $Pt(ligand)_2$ which has no crosslinking ability or a complex lacking 2-phenyl group in its intercalator part which has very small DNA binding ability and so on.

The intercalator-linked monofunctional platinum complexes with no linker, $[PtCl(NH_3)_2(INT)]$ (where INT is for example 9-aminoacridine or chloroquine), were reported to have the ability to bind both intercalatively and covalently [14]. In contrast, the compatibility of crosslinking and intercalative binding has also been clearly evidenced in the water-soluble derivatives with long linker chain between the cisplatin moiety and intercalator [13]. The investigation of water-soluble compounds is necessary to prove the dual functional binding of the present short-linker derivatives. However, our results provide some suggestive information for exploring the new functional intercalator-linked cisplatin drugs.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, a grant from Nippon Itagarasu and Rigaku-Denki and the San-Ei Gen Foundation. The authors also thank the Cancer Chemotherapy Center (Japanese Foundation for Cancer Research) for their antitumor activity testing.

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